Amendment dated December 10, 2008 Reply to Office Action of January 10, 2008

AMENDMENTS TO THE CLAIMS

Listing of Claims:

1. (Currently amended) A method for the microbiological isomerization of alphahydroxycarboxylic acids of the formula I

$$\begin{array}{c} HO_{\mathcal{I}_{1}, \mathbb{N}} H \\ R & CO_{2}H \end{array} \tag{I)}$$

where

R is straight-chain or branched C_2 - C_8 alkyl or C_2 - C_8 alkenyl or - $(CH_2)_n$ -Cyc, where n is an integer of 0 to 4, and Cyc is an unsubstituted or mono- or polysubstituted, mono- or binuclear carbo- or heterocyclic ring,

wherein said method comprises isomerizing a substrate comprising consisting essentially of a first stereoisomeric form stereoisomer of an alpha-hydroxycarboxylic acid of the formula (I) with aid-of an enzyme with alpha-hydroxycarboxylic acid racemase activity and, if appropriate, isolating the resultant isomer mixture or a resultant second stereoisomer, or removing the resultant second stereoisomer from the reaction equilibrium in a reaction medium to obtain a second stereoisomer or an isomer mixture comprising the first stereoisomer and the second stereoisomer,

wherein the enzyme is a lactate racemase with an expanded substrate spectrum, which, in addition to (R)- or (S)-lactate, isomerizes at least one further (R)- or (S)-alphahydroxycarboxylic acid of the formula (I).

2. (Currently amended) A method as claimed in The method of claim 1, wherein the isomerization is effected by converting the substrate with enzyme is a purified enzyme, an enzyme[[-]]containing contained in a cell extract, or in the presence of an enzyme present in intact cells which express at least one the enzyme with alpha hydroxycarboxylic acid racemase activity.

Application No.: 10/560,455 Docket No.: 13111-00027-US

Amendment dated December 10, 2008
Reply to Office Action of January 10, 2008

3. (Currently amended) A method as claimed in The method of claim 1, wherein the enzyme with alpha-hydroxycarboxylic acid racemase activity is isolated from microorganisms of the genus *Lactobacillus* or *Lactococcus*.

- 4. (Currently amended) A-method-as claimed in The method of claim 1, wherein the conversion is carried out in the presence of enzyme is an enzyme present in intact cells of microorganisms of the genus *Lactobacillus* or *Lactococcus* or intact cells of a recombinant microorganism which express the lactate racemase with alpha-hydroxycarboxylic acid racemase activity.
- 5. (Currently amended) A method as claimed in The method of claim 4, wherein the microorganism is selected from the group consisting of L. paracasei, L. delbrueckii, L. sakei and L. oris.
- 6. (Currently amended) A method as claimed in The method of claim 5, wherein the microorganism is selected from the group consisting of the strains *L. paracasei* DSM 20207 (DSM 15755), *L. paracasei* DSM 2649 (DSM 15751), *L. delbrueckii* DSM20074 (DSM 15754), *L. sakei* DSM 20017 (DSM 15753) and *L. oris* DSM 4864 (DSM 15752).
- 7. (Currently amended) A-method as claimed in The method of claim 1, wherein the enzyme isomerizes at least one compound selected from the group consisting of phenyl lactate, 4-fluorophenyl lactate, 2-hydroxy-4-phenylbutyric acid, 2-hydroxy-4-methylpentanecarboxylic acid, 2-hydroxy-3-methylbutyric acid.
- 8. (Withdrawn) A method for screening microorganisms which express an enzyme with alpha-hydroxycarboxylic acid racemase activity, wherein the method comprises growing a lactate-producing or lactate-metabolizing microorganism having racemase activity in the presence of a substrate comprising essentially a stereoisomeric form of an alpha-hydroxycarboxylic acid of the formula (I), and examining the reaction medium for racemization of the substrate.
- 9. (Withdrawn) The method as claimed in claim 8, wherein the microorganisms are of the genus *Lactobacillus* or *Lactococcus*, or recombinant microorganisms which express alphahydroxycarboxylic acid racemase activity.

Application No.: 10/560,455 Docket No.: 13111-00027-US

Amendment dated December 10, 2008 Reply to Office Action of January 10, 2008

10. (Withdrawn) The method as claimed in claim 8, wherein the microorganisms obtained from the screening racemize essentially the stereoisomeric substrate to 1 to 100%.

- 11. (Withdrawn) An alpha-hydroxycarboxylic acid racemase obtained by growing a microorganism selected according to claim 8 which has a positive racemase activity and isolating the alpha-hydroxycarboxylic acid racemase from the culture.
- 12. (Withdrawn) The alpha-hydroxycarboxylic acid racemase as claimed in claim 11, wherein the racemase activity racemizes at least one alpha-hydroxycarboxylic acid of the formula (I) between 1 to 100%.
- 13. (Withdrawn) A nucleic acid encoding at least one alpha-hydroxycarboxylic acid racemase as claimed in claim 11.
- 14. (Withdrawn) An expression vector comprising the nucleic acid as claimed in claim 13 operably linked with at least one regulatory nucleotide sequence.
- 15. (Withdrawn) A recombinant prokaryotic or eukaryotic microorganism comprising at least one nucleic acid as claimed in claim 13.
- 16. (Withdrawn) A method for producing a protein with alpha-hydroxycarboxylic acid racemase activity, wherein the method comprises growing the recombinant prokaryotic or eukaryotic microorganism as claimed in claim 15 and isolating the protein from the culture.
- 17. (Withdrawn) A method for isolating a protein with alpha-hydroxycarboxylic acid racemase activity, wherein the method comprises disrupting a microorganism having a positive racemase activity, removing cell wall fragments and isolating the protein with the desired enzyme activity.
- 18. (Currently amended) The method as claimed in of claim1, wherein the resultant second stereoisomer is essentially removed from the isomer mixture and the remaining part of the isomer mixture is subjected to a further isomerization step.
- 19. (Currently amended) The method as claimed in of claim 1, wherein the resultant isomer mixture is subjected to a <u>subsequent</u> chemical or enzymatic stereoselective subsequent reaction and <u>a reaction mixture is obtained</u>, wherein the reaction mixture obtained is subjected to a further isomerization step.

Application No.: 10/560,455 Docket No.: 13111-00027-US

Amendment dated December 10, 2008 Reply to Office Action of January 10, 2008

20. (Currently amended) The method as elaimed in of claim 1, wherein the isomerization reaction is coupled with a <u>subsequent</u> chemical or enzymatic, enantioselective subsequent reaction, during which the <u>resultant second</u> stereoisomer of the alpha-hydroxycarboxylic acid is removed from the <u>isomerization</u> reaction <u>equilibrium</u> <u>medium</u>.

- 21. (Currently amended) The method as claimed in of claim 19, wherein the <u>subsequent</u> chemical or enzymatic, enantioselective subsequent reaction is an esterification or an amidation of the alpha-hydroxycarboxylic acid.
- 22. (Currently amended) The method as claimed in of claim 20, wherein the <u>subsequent</u> chemical or enzymatic, enantioselective subsequent reaction is an esterification or an amidation of the alpha-hydroxycarboxylic acid.
- 23. (Withdrawn) The method as claimed in claim 8, wherein the microorganisms are selected from the group consisting of *L. paracasei*, *L. delbrueckii*, *L. sakei* and *L. oris*.
- 24. (Withdrawn) The alpha-hydroxycarboxylic acid racemase as claimed in claim 11, wherein the racemase activity racemizes at least one alpha-hydroxycarboxylic acid of the formula (I) between 20 to 100%.
- 25. (Withdrawn) The alpha-hydroxycarboxylic acid racemase as claimed in claim 11, wherein the racemase activity racemizes at least one alpha-hydroxycarboxylic acid of the formula (I) between 50 to 100%.
- 26. (New) The method of claim 1, further comprising isolating the isomer mixture or the second stereoisomer from the reaction medium.
- 27. (New) The method of claim 1, further comprising removing the second stereoisomer from the reaction medium.